Trail Marking Substance of the Texas Leaf-Cutting Ant: Source and Potency

Abstract. *The trail-marking substance of* Atta texana *(Buckley) is formed in the true poison gland and deposited by the sting. This attractant is initially produced by teneral workers. The substance obtained from mature, large workers is the most potent.*

Trail-following behavior is highly developed in the Texas leaf-cutting ant, *Atta texana* (Buckley). This ant marks its trail with a substance that is highly persistent, stable, and perceptible to ants even when it is in minute quantities. It is produced by the true poison gland and deposited by the sting, which is not used for stinging; thus its defense mechanism is used for purposes of communication.

Study of the behavior of a laboratory colony of ants reveals the method of trail marking. When a new supply of green leaves is placed near the nest, scouting workers examine it, and as they return to the nest without forage material, they touch their abdomens to the ground regularly at 2 or 3 mm intervals. Presently, other workers follow this trail, begin cutting leaves, and carry sections to the nest. They mark the trail frequently while carrying the forage, but when returning to the source of supply the marking is sporadic.

In the field, well-defined surface trails may extend several hundred feet to plants being cut. Ants may travel the same trail for months. Trails are used for foraging, never for removal of detritus as in certain other species of *Atta*. Workers forage when surface temperatures of the trail are between 52° and 85°F. Thus, trail activity usually occurs during the day in winter and at night in summer. Activity continues on wet trails, even during light rain, because the odoriferous substance is insoluble in water. A heavy rain disrupts the following of a trail. When a wet or dry trail is broken—as with a scratch of a finger or small stick—traffic is disorganized until the ants replace the scent.

The true poison gland secretes the substance that accumulates in the poison sac. Contents of the Dufour's gland produce no response although this gland is the source of the substance excreted for trail-marking by the myrmicine ants, *Solenopsis* spp. (*1*) and *Pheidole fallax* Mayr (*2*). In *Atta texana*, poison sacs were absent in larvae, empty in pupae, about half full in teneral workers, and turgid in mature workers.

The trail-marking substance is a clear, viscous liquid, soluble in methylene chloride and carbon tetrachloride. It forms a milky-white suspension in acetone, alcohol, or water. When a poison sac is crushed on a glass slide, its contents quickly solidify into a hard, shiny, amorphous material resembling clear fingernail polish.

Artificial trails were made by crushing a single poison sac on the tip of a matchstick and drawing the stick across a sheet of paper. Workers, males, and virgin queens readily followed this path. Crushed heads, thoraxes, abdomens, or contents of the hindgut failed to elicit similar behavior.

We then conducted bioassays to evaluate the potency of the substance from two teneral workers and six mature workers of different sizes. Each poison sac was crushed in 1 ml of carbon tetrachloride and shaken thoroughly. We applied 0.1 ml of the solution to a piece of paper, and described a circle with a diameter of 6 inches. Two lots of ten minor workers (medium and large workers were too excitable for tests) were released in the circle at different times. The number that traveled at least 6 inches on the artificial trail in a period of 5 minutes was used as an index of the potency of the contents of the sac. The lowest concentration that workers could detect was determined by serial dilutions.

Partially full sacs of teneral workers gave a moderate to strong response, but an even stronger reaction was obtained from the turgid sacs of mature workers (Table 1). Sacs from the biggest mature workers tended to have the highest potency. Sacs from pupae produced no response.

Contents of poison sacs crushed on matchsticks or in carbon tetrachloride and kept at room temperature had high potency after 5 months. Contents of poison sacs held at −12°C for 5 months were fully potent. The contents did not freeze. Poison sacs remained intact in dead workers after other abdominal parts had decomposed to a soupy mass; the contents of the sacs were fully potent.

Table 1. Potency of odoriferous substance deposited by teneral workers and older workers of various sizes. The response is computed from two replications of ten workers each, and the number in the table represents the number of workers following the trail over a distance of 6 inches or more. The contents of one poison sac were taken up in 1 ml of CCl_4 and serially diluted (by powers of 10) in CCl_4.

Worker length (mm)	Poison sac (length × width) (mm)	Response					
		1	10	10^2	10^3	10^4	10^5
Teneral workers (sacs partially full)							
5.0	0.30 × 0.20	13	1	1	2	0	0
5.0	.35 × .25	9	5	0	1	0	0
Mature workers (sacs turgid)							
2.2	.10 × .10	17	1	0	0	0	0
2.3	.10 × .05	16	1	0	0	0	0
5.0	.50 × .40	20	18	6	0	0	0
6.0	.60 × .40	19	18	3	0	2	0
8.0	.70 × .60	14	10	12	0	1	0
9.0	.80 × .55	17	14	9	1	0	0

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Myoglobin: Inherited Structural Variation in Man

Abstract. *Ultrafiltrates of human myoglobin from 200 individuals were examined by starch-gel electrophoresis. Only two variants, $Mb^{Aberdeen}$ and $Mb^{Annapolis}$, of the commonly occurring myoglobin Mb^{+} were encountered. Each variant is apparently inherited as an autosomal codominant. Heterozygotes for either mutation lack apparent muscular disease. Combined electrophoresis and chromatography of $Mb^{Annapolis}$ indicate a loss of arginine at a position near the C-terminal portion of the molecule.*

Although considerable genetic variation occurs among the hemoglobins of man and other species, no such variation has been reported for myoglobin. We have discovered two examples of inherited structural variation of human myoglobin. The detection and characterization of additional myoglobin mutants